Carbohydrate Polymers 82 (2010) 1097–1101

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Carbohydrate composition of eucalyptus, bagasse and bamboo by a combination of methods

Ericka F. Alves[a], Samar K. Bose[a], Raymond C. Francis[a,*], Jorge L. Colodette[b], Mikhail Iakovlev[c], Adriaan Van Heiningen[c,d]

[a] Department of Paper and Bioprocess Engineering, State University of New York (SUNY), College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210, USA
[b] Department of Forestry Engineering, Federal University of Viçosa, Viçosa, MG 36570-000, Brazil
[c] Department of Forest Products Technology, Aalto University, P.O. Box 6300, FI-00076 Aalto, Espoo, Finland
[d] Department of Chemical and Biological Engineering, University of Maine, 5737 Jenness Hall, Orono, ME 04469-5737, USA

ARTICLE INFO

Article history:
Received 13 April 2010
Received in revised form 11 June 2010
Accepted 18 June 2010
Available online 7 July 2010

Keywords:
Glucan
Xylan
Uronic acids
Sulfuric acid hydrolysis
Acidic methanolysis

ABSTRACT

The carbohydrate composition of lignocellulosic biomass is normally obtained by the quantification of monomers after two stages of hydrolysis in H_2SO_4. A new two-stage hydrolysis protocol has recently been developed but so far only results for temperate hardwoods have been reported. In this investigation the new protocol was used in the analysis of depithed sugarcane bagasse, *Eucalyptus grandis* and bamboo. The results were compared to those from the traditional hydrolysis protocol. Sugar monomers were quantified by ^{1}H NMR and high performance liquid chromatography (HPLC) and there were no significant differences between the two techniques. The new and traditional hydrolysis protocols gave nearly identical results for the bagasse and bamboo samples both of which contain <0.10 uronic acids per xylose unit. However, the new protocol gave a higher xylan yield (13.2% vs. 12.1%) for the *E. grandis* that contained >0.20 uronic acids per xylose units. For both hydrolysis protocols, summative analyses in the range of 97.5–101.0% were obtained for the three biomass samples. However, the range was narrower (98.6–99.5%) for the new hydrolysis protocol coupled with ^{1}H NMR analysis.

1. Introduction

Most quantification of carbohydrate monomers from biomass samples start with a hydrolysis procedure close to the one first reported by Saeman, Moore, Mitchell, and Millet (1954). That protocol calls for 1 h of primary hydrolysis (PH) in 72% H_2SO_4 at 30 °C followed by dilution to 4% H_2SO_4 and secondary hydrolysis (SH) for 1 h at 121 °C. In an earlier paper it was demonstrated that SH treatment in 40% H_2SO_4 at 80 °C for 50–70 min afforded higher xylose yields for temperate hardwoods and with a much lower standard deviation than is typically reported for the Saeman et al. method (Bose et al., 2009). Similar to data cited in the Bose et al. paper for sugar maple (*Acer saccharum*) and aspen (*Populus tremuloides*), the literature contains a relatively wide variation in xylan content (calculated from xylose yield) for depithed sugarcane bagasse when protocols close to that of Saeman et al. were used. Rabelo, Filho, and Costa (2008) obtained glucan and xylan contents of 39.6% and 19.7%, respectively, while Aguilar, Ramírez, Garrote, and Vázquez (2002) obtained values of 38.9% and 20.6% and Neureiter, Danner, Thomasser, Saidi, and Braun (2002) obtained values of 40.2% and 22.5%. While the highest of the three glucan contents is only 3.3% greater than the lowest, the corresponding value for xylan content is 14.2% (22.5/19.7 = 1.142).

One of the primary objectives of this research was to see if consistently high xylan contents would be obtained for depithed bagasse when SH is performed in 40% H_2SO_4 at 80 °C. A second objective was to compare the results obtained with this hydrolysis protocol plus sugar analysis by ^{1}H NMR to the Saeman et al. hydrolysis protocol coupled with sugar analysis by HPLC. A third objective was to see if summative analyses close to 100% could be achieved for depithed bagasse, bamboo and *Eucalyptus grandis*. In the earlier research (Bose et al., 2009), uronic acid contents were estimated but they were determined by acidic methanolysis in this investigation. The research program was conducted in the laboratories of three universities in the USA, Brazil and Finland.

2. Materials and methods

2.1. Biomass samples

E. grandis (Mogi Guaçu, São Paulo, Brazil) and *Bambusa vulgaris* (Coelho Neto, Rio de Janeiro, Brazil) chips were obtained

* Corresponding author. Tel.: +1 315 470 6525; fax: +1 315 470 6945.
E-mail addresses: francis@esf.edu, francis@syr.edu (R.C. Francis).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.06.038

from Brazilian pulp manufacturing facilities. Nodal sections of the *B. vulgaris* were removed before chipping. Depithed sugarcane (*Saccharum officinarum*) bagasse (Sample A) was obtained from a pulp mill located in Jujuy Province, Argentina. Sugarcane (*S. officinarum*) bagasse Sample B, already depithed, was obtained from a sugar factory in KwaZulu-Natal, South Africa. All four samples were extracted with ethanol/toluene in accordance with Tappi Method T 204 om-88 (Tappi, 1988a,b) before all analyses were initiated.

2.2. Hydrolysis of milled biomass followed by ^{1}H NMR analysis

Extractive-free biomass meal (0.50 g, oven dried or OD basis) was added to a 50 ml centrifuge tube and placed in a water bath at 25 °C. Then 16 ml of 72% H_2SO_4 (specific gravity 1.634) was added and carefully kneaded into the particles using a glass rod. The slurry was allowed to sit in the water bath at 25 ± 1.0 °C for 2 h with mixing every 15 min. After primary hydrolysis, water (21 ml) was added to the slurry and the tubes sealed (now 40 wt% H_2SO_4), shaken, and placed in a water bath at 80 ± 1.0 °C for 60 min. The tubes were shaken occasionally to homogenize the slurry. After 60 min of hydrolysis treatment, the tubes were removed from the water bath and chilled in an ice bath. They were stored overnight in a refrigerator (4 °C) and lignin precipitation occurred. A recorded mass of the supernatant was taken and the internal standards were added. The solution was then analyzed by ^{1}H NMR spectroscopy as soon as possible. All the detailed information about the quantification of the relative molar concentration of sugars and other compounds resulting from wood hydrolysis is described by Bose et al. (2009). The use of glucosamine (GluN) as a back-up internal standard was not recorded in the earlier paper (Bose et al., 2009). Trimethylamine hydrochloride (TMA) was the primary internal standard but its concentration decreased when there was a long delay (>24 h) between sample preparation and ^{1}H NMR analysis. In such cases GluN (added at 1.9×10^{-4} mol/g) was used as an internal standard to determine the TMA concentration at the time of analysis. This research was performed at SUNY College of Environmental Science and Forestry, Syracuse, NY.

2.3. Hydrolysis of milled biomass followed by HPLC analysis

Extractive-free biomass meal (300 mg, OD basis) was quantitatively transferred to a test tube (60 mm × 1.5 mm) and 3 ml of 72% H_2SO_4 were added and kneaded into the particles. The slurry was allowed to sit in the water bath at 30 °C for 1 h with occasional mixing. The slurry was then transferred into a penicillin bottle containing 84 ml of deionized water and the flask was sealed with a rubber stopper and aluminum seal. The bottle was placed in an autoclave calibrated at 118 °C for 1 h. The slurry was then filtered through a regenerated cellulose membrane (0.45 μm) and the filtrate quantitatively transferred to a 250 ml volumetric flask and subsequently analyzed for sugars.

The sugar solution (50 ml) and 1 ml of erythrol (1.0 g/l) were added to a beaker and the pH was adjusted to 5.3 with a saturated solution of barium hydroxide. The mixture was then centrifuged at 5000 rpm for 2.5 min then analyzed by HPLC using instrument model SCL-10A, equipped with a refractive index detector, RID-10A, and columns HPX 87P (7.8 mm × 300 mm) and SCR 101P (7.9 mm × 300 mm) coupled at 80 °C. The samples were analyzed with deionized water as eluent at a flow rate of 0.4 ml/min for 70 min. Correlations between peak areas and concentrations were determined for authentic samples of glucose, xylose, galactose, arabinose, mannose, etc. These correlations were used to determine the concentration of sugars in the hydrolyzates. This research was performed at the University of Viçosa, Minas Gerais, Brazil.

2.4. Acidic (HCl) methanolysis followed by gas chromatographic (GC) analysis

A known mass close to 10 mg of the dry biomass was transferred to a pear shaped vessel and dried in a vacuum oven (40 °C, 70 mbar for 1 h). Two (2) milliliters of the methanolysis reagent (2 M HCl in methanol, prepared by adding acetyl chloride to anhydrous methanol) was added and the vessel was tightly closed and put into an oven (100 °C for 5 h). The vessels were shaken every 1 h to ensure uniform hydrolysis. After cooling to room temperature the vessels were opened and 200 μl of pyridine were added to neutralize the excess of HCl followed by addition of 1 ml of the standard solution (0.1 mg/ml sorbitol in methanol). The methanol was then evaporated in a stream of nitrogen. After that the vessels were put in the vacuum oven (40 °C, 70 mbar for 30 min) to obtain dried samples. The samples were then dissolved in 0.5 ml anhydrous pyridine (10 min in ultrasonic bath) and silylated by adding 250 μl of the silylating mixture (BSFTA + 5% TMC). The silylation was done overnight (10–15 h). The samples were then centrifuged and transferred to the GC vials. The analysis was done with a Shimadzu GC-17A gas chromatograph using NB-30 capillary column (length 30 m, internal diameter 0.32 mm). The temperature program: 2 min at 100 °C,

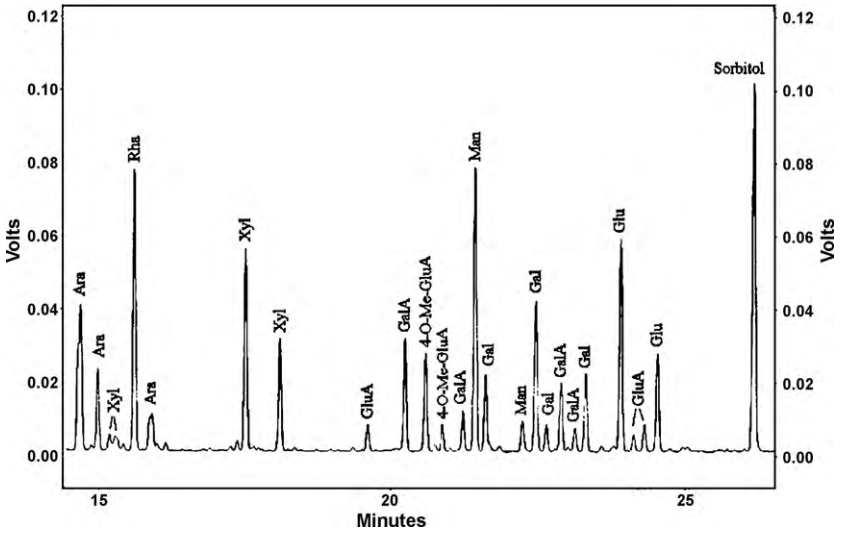

Fig. 1. A typical acid methanolysis chromatogram.

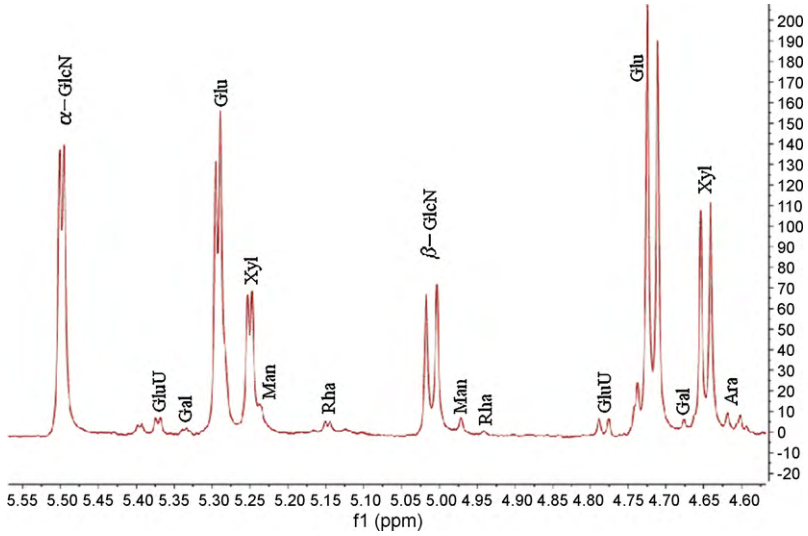

Fig. 2. ^{1}H NMR spectrum for sugars in the hydrolyzate of a hardwood; this spectrum is typical for biomass containing a high concentration of xylan (>10 wt%). Acronym GlcN stands for glucosamine an internal standard.

4 °C/min to 220 °C and 2 min at 220 °C. The sample (1 μl) was introduced via split injector (290 °C). The carrier gas was hydrogen and FID detection (290 °C) was used. A typical chromatogram is shown in Fig. 1. These analytical protocols are similar to those of Sundberg, Sundberg, Lillandt, and Holmbom (1996) and Bertaud, Sundberg, and Holmbom (2002) but with some minor modifications. This research was performed at Helsinki University of Technology (now Aalto University), Espoo, Finland.

2.5. Other analyses

Lignin content was determined as previously described (Bose et al., 2009) while ash content was determined by Tappi Method T 211 om-93 (Tappi, 1993).

3. Results and discussion

Descriptions of the HPLC technique and examples of typical chromatograms for biomass hydrolyzates can easily be found in the literature. However, an excellent description of the technique is provided by Kaar, Cool, Merriman, and Brink (1991) who used it to determine the carbohydrate composition of nine common North American wood species in a later publication (Kaar & Brink, 1991). Excellent and detailed results on polysaccharide characterization using the HPLC method were also reported by Wallis, Wearne, and Wright (1996).

A typical spectrum resulting from ^{1}H NMR analysis of biomass hydrolyzate (20–40 wt% H_2SO_4) is shown in Fig. 2. It can be seen that the sugar peaks are well resolved and that the peaks for glucose and xylose are dominant. As previously discussed (Bose et al., 2009), the peaks between 4.60 and 5.05 ppm are for the C1-β protons while those between 5.15 and 5.55 ppm are for the C1-α protons. The peaks for other detectable compounds and internal standards fall outside the range of that shown in Fig. 2. However, the approximate locations of those peaks are already reported (Bose et al., 2009).

The peaks assigned as GluU were observed with an authentic sample of glucuronic acid. However, they do not show up consistently when hydrolyzates are analyzed and as such we do not use these peaks to estimate the uronic acid content of biomass samples. The acid-catalyzed cleavage of the glucuronosyl linkage between 4-O-methylglucuronic acid (Me-GluU) and a xylose trimer (Me-GluU(1 → 2)α-xylose) as well as the stability of released monomers was previously investigated (Bertaud et al., 2002). When the HCl concentration was maintained close to 2 M (in methanol) for 3 h at 100 °C there was a high rate of hydrolysis of Me-GluU but ~75% of it degraded (Bertaud et al., 2002). It is possible that our PH and SH conditions afforded almost complete hydrolysis of GluU and Me-GluU but degraded a majority of the uronic acid monomers generated. In the new protocol, PH is performed for 2 h in 72% H_2SO_4 instead of the 1.0 h suggested by Saeman et al. (1954) and this is followed by a 60 min treatment in 40% H_2SO_4 instead of 4.0% H_2SO_4 recommended by Saeman et al. (1954). A sample to sample variation in the rate of degradation of uronic acids would explain the variation in the uronic acids to xylose that is observed in our ^{1}H NMR spectra. On many occasions the uronic acid peaks are not observed. Also, the reproducibility of arabinose, mannose, galactose and Rhamnose concentrations ranges from poor to average for hardwoods (Bose et al., 2009).

3.1. Xylan content of bagasse by NMR, HPLC and methanolysis

The ^{1}H NMR results for Sample A (South America) and Sample B (South Africa) are presented first in Table 1. The ^{1}H NMR sugar results along with lignin, ash and uronic anhydride contents afforded summative analyses of 99.2% and 99.1% for the two samples. The samples were almost equal in glucan content at 41.3% and 41.4%, respectively. The content of lignin, glucan and xylan for the two samples are close to 23.1% lignin, 41.7% glucan and 24.7% xylan reported for the U.S. National Institute of Standards and Technology (NIST) bagasse standard reference material #8491 (Scurlock, Dayton, & Hames, 2000). Although both of our xylan contents are higher than the three values reported in Section 1, the value for Sample A (24.9%) was approximately equal to the 23.8% value obtained by the HPLC method (Table 1). The HPLC results did not include xylose dehydrated to furfural. The furfural yield for the Saeman et al. (1954) hydrolysis protocol is estimated at ~0.05 mole/mole xylose (to be discussed later) and multiplication of the 23.8% xylan content by 1.05 results in a value of 25.0%. The 95% confidence interval data in Tables 1 and 2 were estimated based on extensive data collected in the SUNY and University of Viçosa laboratories plus data in the published literature where many replicates were used for glucan, xylan and lignin analyses (Bose et al., 2009; Kaar et al., 1991; Tappi Method T 222 om-88, 1988). A significant difference between the HPLC and ^{1}H NMR methods was not expected with bagasse for two reasons. First, the researchers

Table 1
Chemical composition (wt%) of extractive-free sugarcane bagasse by different techniques; Bagasse-A (South American), -B (South African).

	Bagasse-A NMR	Bagasse-B NMR	Bagasse-A HPLC	Bagasse-A Methanolysis
% Glucan	41.3 ± 0.4[a]	41.4 ± 0.4[a]	43.1 ± 0.3[a]	
% Xylan	24.9 ± 0.2[a]	23.9 ± 0.2[a]	23.8 ± 0.3[a] 25.0[b]	20.1[c]
% Galactan	0.6	0.6	0.4	
% Mannan	–	–	0.3	
%Arabinan	1.7	2.4	1.5	
% Lignin	23.2 ± 0.3[a]	23.9 ± 0.3[a]	23.2 ± 0.3[a]	
% Acetyl	3.0	2.8	3.0[d]	
% Uronics [e]	1.2[f]	1.2[f]	1.2[f]	1.2
% Me-GluU	0.8[f]	0.8[f]	0.8[f]	0.8
% Ash	2.5	2.1	2.5	
% Total	99.2	99.1	99.8 101.0[b]	

[a] Average for 2–4 samples plus estimated 95% confidence interval (see text).
[b] Corrected for xylose dehydrated to furfural (see text).
[c] Excluding xylose converted to furfural.
[d] NMR value.
[e] Glucuronic and galacturonic acids.
[f] Methanolysis value; calculated as anhydrides.

at the University of Viçosa who performed the HPLC analysis are highly skilled and have many years of experience with the analytical protocols. Second, bagasse contains a low number of uronic acids groups. The 1 → 2 linkage between Me-GluU and xylose units in xylan are known to be resistant to mild acid hydrolysis (Bertaud et al., 2002; Whistler & Richards, 1958). While temperate hardwoods contain ~0.15 uronic acids per xylose unit (Kaar & Brink, 1991) and some eucalypti contain >0.20 (Magaton, 2008; Wallis et al., 1996), the reported value for sugarcane bagasse is only ~0.05 uronic acids per xylose unit (Brienzo et al., 2009). When the Saeman et al. (1954) hydrolysis protocol is used, lower than expected xylose yields are frequently reported for hardwoods as discussed by Bose et al. (2009). Incomplete cleavage of the glucuronosyl linkages between uronic acids and xylose is a probable cause.

The methanolysis method gave a xylose yield that was ~19% lower than that obtained by ^{1}H NMR for Sample A (Table 1). However, once again the xylose converted to furfural was not quantified by methanolysis. If the uronic acids by methanolysis is correlated with xylan from ^{1}H NMR an uronic acids to xylose ratio of 0.06 is obtained. This value is close to the 0.05 ratio reported by Brienzo et al. (2009). The molecular weights for xylose, galacturonic acid (Ga1U), GluU and Me-GluU as anhydro sugars or anhydrides are 132, 176, 176 and 190, respectively.

3.2. Xylan content of E. grandis and bamboo

The results are presented in Table 2 and those for *E. grandis* will be discussed first. The repeatability for glucose and xylose yields was excellent for both the ^{1}H NMR and HPLC methods. The ^{1}H NMR method afforded duplicate glucan contents of 46.4% and 46.6% while the corresponding values were 46.6% and 46.8% for the HPLC method. The repeatability was also excellent for xylan content with values of 13.1% and 13.2% for ^{1}H NMR and 11.3% and 11.6% by HPLC. If the uronic acids content from methanolysis is combined with the xylan content from ^{1}H NMR then an uronic acids to xylose ratio of 0.20 is obtained. This ratio is close to the 0.22 value obtained by Magaton (2008) who isolated O-acetyl-4-O-methylglucuronoxylan (AMX) from *E. grandis* and analyzed it by acidic methanolysis. The xylan yield determined by methanolysis was 10.4% in the present study. As noted by Bertaud et al. (2002) the methanolysis method underestimates the true uronic acid content of biomass due to incomplete hydrolysis of the glucuronosyl linkage and partial degradation of the uronic acid generated. Magaton (2008) isolated AMX by extraction of *E. grandis* with 24% KOH. The sample was soluble in D_2O and it was analyzed by ^{1}H NMR without being hydrolyzed by a mineral acid solution. The ^{1}H NMR results indicated an uronic acids to xylose ratio of 0.28. Five other eucalypti (*E. dunni*, *E. globulus*, *E. nitens*, *E. urograndis* and *E. urophylla*) were analyzed by ^{1}H NMR and their uronic acids to xylose ratio varied from 0.19 to 0.26 with an average of 0.23 (Magaton, 2008).

A probable explanation for the higher xylose yield using the ^{1}H NMR method in the present study is that its hydrolysis protocol afforded a more complete hydrolysis of the glucuronosyl linkages than the Saeman et al. protocol that was used with the HPLC method. The harsher conditions used in the ^{1}H NMR hydrolysis protocol (2 h of PH in 72% H_2SO_4 at 25 °C followed by SH in 40%

Table 2
Chemical composition (wt%) of extractive-free *E. grandis* and bamboo by NMR and HPLC; samples from South America.

	Eucalyptus-NMR	Eucalyptus-HPLC	Bamboo-NMR	Bamboo-HPLC
% Glucan	46.5 ± 0.4[a]	46.7 ± 0.3[a]	49.4 ± 0.4[a]	50.4 ± 0.3[a]
% Xylan	13.2 ± 0.2[a]	11.5 ± 0.3[a] 12.1[b]	18.7 ± 0.2[a]	18.7 ± 0.3[a] 19.6[b]
% Galactan	1.4	1.2	0.4	0.4
% Mannan	0.9	1.0	0.5	0.3
% Arabinan	1.5	0.5	1.1	1.2
% Lignin	29.2 ± 0.3[a]	29.2 ± 0.3[a]	23.4 ± 0.3[a]	23.4 ± 0.3[a]
% Acetyl	2.8	2.8[c]	2.4	2.4[c]
% Uronics	2.1[d]	2.1[d]	0.5[d]	0.5[d]
% Me-GluU	1.6[d]	1.6[d]	0.7[d]	0.7[d]
% Ash	0.3	0.3	1.5	1.5
% Total	99.5	96.9 97.5[b]	98.6	99.5 100.4[b]

[a] Average for 2–4 samples plus estimated 95% confidence interval.
[b] Corrected for xylose dehydrated to furfural (see text).
[c] NMR value.
[d] Methanolysis value; calculated as anhydrides.

H_2SO_4 for 1 h at 80 °C) probably degraded most of the liberated uronic acids.

When the Saeman et al. hydrolysis protocol was used for sugar maple and aspen the ratio of furfural to xylose in the hydrolyzates were 0.07 and 0.01, respectively (Mittal, Scott, Amidon, Kiemle, & Stipanovic, 2009). If an average of 0.05 is assumed then the HPLC xylose yield can be increased by 5% from 11.5% to 12.1%. In light of nearly identical results for bagasse Sample A, it is unlikely that the 9% higher xylose yield (13.2% vs. 12.1%) for the new hydrolysis protocol was due to a difference in quantification methodologies, i.e. ^{1}H NMR versus HPLC.

Very similar results were obtained by the ^{1}H NMR and HPLC methods for the bamboo another biomass with a low uronic acids content (Table 2). The xylan content by HPLC was actually 5% higher than by ^{1}H NMR. The uronic acids to xylose ratio was 0.05 when the uronic acids determined by methanolysis was correlated with xylan by ^{1}H NMR. The xylan content determined by methanolysis was 15.5%.

4. Conclusions

A new hydrolysis protocol has been developed to convert carbohydrate polymers in biomass to their monomeric constituents. However, results have only been reported for temperate hardwoods so far (Bose et al., 2009). This research investigated three different biomass samples that are usually associated with tropical and sub-tropical regions, i.e. sugarcane bagasse, eucalyptus, and bamboo. The new hydrolysis protocol that consists of 2 h of primary hydrolysis in 72% H_2SO_4 at 25 °C followed by 1 h SH in 40% H_2SO_4 at 80 °C gave nearly identical results to the well established hydrolysis protocol of Saeman et al. (1954) for bagasse and bamboo. The new protocol was coupled with ^{1}H NMR analysis of monomers while the traditional hydrolysis protocol was coupled with HPLC analysis. The two analytical techniques (^{1}H NMR and HPLC) appear to give very similar values for glucose and xylose. When the *E. grandis* was analyzed, the new hydrolysis protocol gave a xylan content that was 9% (13.2 wt% vs. 12.1 wt%) higher than for the Saeman et al. (1954) method. Unlike the bagasse and bamboo that had uronic acids to xylose ratios <0.10, the *E. grandis* had a ratio >0.20. The $1 \rightarrow 2$ linkages of Me-GluU to xylose units in xylan are known to be resistant to acidolysis (Bertaud et al., 2002; Whistler & Richards, 1958). It appears as if the new hydrolysis protocol is more efficient at cleaving those glucuronosyl linkages thus affording a higher yield of xylose monomers. The new hydrolysis protocol coupled with ^{1}H NMR analysis afforded summative analyses that fell in a narrow range (98.6–99.5%) for the two bagasse samples plus *E. grandis* and bamboo. The Saeman protocol coupled with HPLC afforded values of 101.0% for one of the bagasse samples and 100.4% for the bamboo. However, a summative analysis of only 97.5% was obtained for the *E. grandis*.

Acknowledgements

We are most grateful to Andritz Inc., the member companies of the Empire State Paper Research Associates (ESPRA), CAPES/Fulbright Doctoral Program (Ericka Alves) for their financial support (Syracuse, USA) and to Suzano, Ledesma and Portella Pulp and Paper Companies for providing the South American eucalypt, bagasse and bamboo samples. We are thankful to Mr. David J. Kiemle for performing the ^{1}H NMR analyses in Syracuse, NY and Dr. Andrej Pranovich, Abo Akademy University, Turku, Finland for his advice on improving the acid methanolysis method at Aalto University, Espoo, Finland.

References

Aguilar, R., Ramírez, J. A., Garrote, G., & Vázquez, M. (2002). Kinetic study of the acid hydrolysis of sugar cane bagasse. *Journal of Food Engineering*, *55*, 309–318.

Bertaud, F., Sundberg, A., & Holmbom, B. (2002). Evaluation of acid methanolysis for analysis of wood hemicelluloses and pectins. *Carbohydrate Polymers*, *48*, 319–324.

Bose, S. K., Barber, V. A., Alves, E. F., Kiemle, D. J., Stipanovic, A. J., & Francis, R. C. (2009). An improved method for the hydrolysis of hardwood carbohydrates to monomers. *Carbohydrate Polymers*, *78*, 396–401.

Brienzo, M., Carvalho, W., & Milagres, A. M. F. (2009). Xylooligosaccharides production from alkali-pretreated sugarcane bagasse using xylanases from *Thermoascus aurantiacus*. *Applied Biochemistry and Biotechnology* (Online article, Humana Press).

Kaar, W. E., & Brink, D. L. (1991). Summative analysis of nine common North American woods. *Journal of Wood Chemistry and Technology*, *11*, 479–494.

Kaar, W. E., Cool, L. G., Merriman, M. M., & Brink, D. L. (1991). The complete analysis of wood polysaccharides using HPLC. *Journal of Wood Chemistry and Technology*, *11*, 447–463.

Magaton (2008). *Comportamento e caracterização de xilanas durante a polpação kraft de eucalipto*. Ph.D. Thesis. Brazil: Universidade Federal de Minas Gerais (UFMG).

Mittal, A., Scott, G. M., Amidon, T. E., Kiemle, D. J., & Stipanovic, A. J. (2009). Quantitative analysis of sugars in wood hydrolyzates with ^{1}H NMR during the autohydrolysis of hardwoods. *Bioresource Technology*, *100*, 6398–6406.

Neureiter, M., Danner, H., Thomasser, C., Saidi, B., & Braun, R. (2002). Dilute-acid hydrolysis of sugarcane bagasse at varying conditions. *Applied Biochemistry and Biotechnology*, *98–100*, 49–58.

Rabelo, S. C., Filho, R. M., & Costa, A. C. (2008). A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production. *Applied Biochemistry and Biotechnology*, *148*, 45–58.

Saeman, J. F., Moore, W. E., Mitchell, R. L., & Millet, M. A. (1954). Techniques for the determination of pulp constituents by quantitative paper chromatography. *Tappi Journal*, *37*, 336–343.

Scurlock, J. M. O., Dayton, D. C., & Hames, B. (2000). Bamboo: An overlooked biomass resource? *Biomass and Bioenergy*, *19*(4), 229–244.

Sundberg, A., Sundberg, K., Lillandt, C., & Holmbom, B. (1996). Determination of hemicelluloses and pectins in wood and pulp fibres by acid methanolysis and gas chromatography. *Nordic Pulp and Paper Research Journal*, *11*(4), 216–219.

Tappi. (1988a). *Solvent extractives of wood and pulp. Tappi Method T 204 om-88*. Atlanta, GA: Tappi Press.

Tappi. (1988b). *Acid-insoluble lignin in wood and pulp. Tappi Method T 222 om-88*. Atlanta, GA: Tappi Press.

Tappi. (1993). *Ash in wood and pulp. Tappi Method T 211 om-93*. Atlanta, GA: Tappi Press.

Wallis, A. F. A., Wearne, R. H., & Wright, P. J. (1996). Analytical characteristics of plantation eucalypt woods relating to kraft pulp yield. *Appita Journal*, *49*, 427–432.

Whistler, R. L., & Richards, G. N. (1958). Uronic acid fragments from slash pine *(Pinus elliottii)* and their behavior in alkaline solution. *Journal of American Chemical Society*, *80*, 4888–4891.